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CED
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Increased ratio of FoxP3+ regulatory T cells/CD3+ T cells in skin lesions in drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms

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Summary

Background. Drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS) is a severe drug eruption accompanied by multiorgan disorders. Several unique aspects of DIHS/DRESS, including herpesvirus reactivation, liver dysfunction and hypogammaglobulinaemia, have similarities to graft-versus-host disease (GVHD).

Aim. In this study, we focused on the dynamics of regulatory T cells (Tregs) infiltrating into the skin lesions of DIHS/DRESS and GVHD.

Methods. Skin biopsies were taken from patients with DIHS/DRESS, GVHD, or maculopapular drug eruption. Tregs were detected using immunostaining with anti-FoxP3.

Results. The ratio of FoxP3+ T cells to CD3+ T cells was significantly higher in the skin lesions of patients with DIHS/DRESS than in those of patients with GVHD, and was positively correlated with the number of days from disease onset in the acute phase.

Conclusions. The dynamics of Tregs in skin lesions are different between DIHS/DRESS and GVHD, despite there being many similarities between these conditions.

Introduction

Drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS) is a severe drug eruption accompanied by multiorgan disorders.[1] It may be related to reactivation of human herpesvirus (HHV), especially HHV-6,[2–4] and to mild epidermal injury, in contrast to other severe adverse cutaneous drug reactions such as toxic epidermal necrosis (TEN) and Stevens–Johnson syndrome (SJS). However, the mechanisms of HHV reactivation and development of drug rashes are currently unknown. DIHS/DRESS has several notable features, such as delayed onset, worsening of clinical symptoms even after withdrawal of the causative drug, hypogammaglobulinaemia,[5] reactivation of latent HHV during the acute stage of the disease, and autoimmune complications developing as short-term or long-term sequelae, such as autoimmune thyroiditis, positive reaction of antinuclear antibodies and fulminant type 1 diabetes mellitus.[6,7] Many aspects of this syndrome suggest close similarities between DIHS/DRESS and graft-versus-host disease (GVHD). We and other researchers have also revealed a relationship between HHV-6 reactivation and rash/GVHD after allogeneic

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stem cell transplantation,[8,9] Various complications frequently occurring in GVHD, such as autoimmune disease,[10,11] are frequently observed during the course of DIHS/DRESS, even long after its clinical resolution. However, there are clinical and histological differences between DIHS/DRESS and GVHD; for example, interface dermatitis and apoptotic keratinocytes can be observed in both DIHS/DRESS and GVHD, but are more severe in the latter.

Recently, much attention has been focused on regulatory T cells (Tregs) and their roles in drug eruption/GVHD. However, the dynamics of Tregs in the skin lesions in DIHS/DRESS and GVHD are not fully understood. In this study, we focused on the dynamics of Tregs infiltrating into the skin, one of the major target organs in DIHS/DRESS and GVHD, to examine the involvement of Tregs in the development of DIHS/DRESS and GVHD skin lesions.

Methods

The study was approved by the medical ethics committee of Nara Medical University, and all patients gave informed consent.

Patients and samples

Our study consisted of three groups of patients: patients with DIHS/DRESS (n = 12), patients with acute GVHD (n = 12) and patients with maculopapular drug eruption (MDE) (n = 18). The eliciting drugs had been withdrawn by the time of diagnosis of DIHS/DRESS or drug eruption in all patients.

The DIHS/DRESS group consisted of 12 patients (5 men, 7 women; median age 59 years, range 13–75) who were enrolled consecutively during the period April 2003 to November 2011. The profiles of these patients are shown in Table 1. Diagnosis of DIHS/DRESS was based on criteria established by a Japanese consensus group[12] and by RegiSCAR (European Registry of Severe Cutaneous Adverse Reactions).[13] Reactivation of HHV, including HHV-6 and HHV-7, was demonstrated by an increase in the titre of the specific serum IgG antibody and/or DNA levels in whole blood as detailed below. Skin biopsies were also taken from areas of maculopapular erythema in this group.

Table 2 details the characteristics of 12 consecutive patients with clinical signs of acute GVHD (3 men, 9 women; median age 52 years, range 7–66) who received allogeneic stem cell transplantation for haematological malignancy during the period November 2002 to August 2011. All 12 patients had received standard prophylaxis (ciclosporin in 10 patients and mycophenolate mofetil in 2 patients) prior to transplantation. Skin biopsies were taken from areas of erythematous maculopapular rash in all 12 patients, which were clinically graded according to standard criteria.[14,15]

The final group consisted of 18 patients (10 men, 8 women; median age 61 years, range 32–81). Skin biopsies were also taken from areas of cutaneous rash of patients without allografts or DIHS/DRESS (n = 18), which was clinically and histopathologically considered to be an MDE.

Assessment of herpesvirus DNA

DNA levels were assessed by PCR. DNA was extracted from whole blood using a commercial kit (QIAamp DNA Blood Mini-kit; Qiagen Inc., Tokyo, Japan) in accordance with the manufacturer's instructions, and then used for PCR. For assessment of HHV-6 and HHV-7 DNA levels in peripheral blood, real-time PCR was performed as described in a previous report,[16] and results expressed as viral DNA genome equivalents per 1 mL of whole blood. In DIHS/DRESS, HHV-6 DNA is usually detected during days 14–21 after the onset of skin eruption, whereas it usually increases in accordance with the skin eruption in GVHD, as described previously.[9]

Immunohistochemistry

Tissues were fixed in formalin, embedded in paraffin wax, and cut into sections 4 μm thick. Immunostaining was performed using anti-CD3 (code A0452; Dako, Glostrup, Denmark) polyclonal antibody, and anti-FoxP3 (clone 236 A/E7; BD Biosciences Inc., San Jose, CA, USA), anti-CD4 (NCL-CD4-368, clone 4B12) and anti-CD8 (NCL-C8-295, clone 1A5) (both Novocastra Ltd, Newcastle upon Tyne, UK) monoclonal antibodies as primary antibodies. Biotinylated antimouse IgG was used as secondary antibody, and bound antibody was evaluated using streptavidin-biotinylated peroxidase complex. After washing, sections were exposed to the chromogen and counterstained with haematoxylin. The numbers of immunostained cells in the dermis were counted in five high-power fields (HPF) and expressed as the mean number. The ratios of FoxP3+ Tregs and CD4+ T cells, and the ratio of CD8+ T cells to CD3+ T cells in the dermis were then calculated.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using the Student t-test. Pearson

Table 1 Characteristics of patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms.

Patient	Age, years/sex	Causative drug	Viral reactivation	Viral DNA loads* (in whole blood) or titres	Time from disease onset to skin biopsy, days	Immunosuppressive treatments at the time of skin biopsy	Time between skin biopsy and viral reactivation, days	FoxP3+/ CD3+ cells in skin lesions,%	Skin rash	Eosinophils, per μL	Liver dysfunction, AST/ALT, IU/L
1	62/M	Carbamazepine	HHV-7	1.2×10^4	5	None	−1	14.7	Maculopapular erythema	980	286/723
2	68/F	Carbamazepine	HHV-6	8.8×10^3	15	None	2	17	Maculopapular erythema	1560	34/45
3	75/F	Allopurinol	HHV-6, HHV-7	1.3×10^3 (HHV-6)	13	None	3	27.2	Maculopapular erythema, purpura	1200	57/84
4	61/F	Salazosulfapyridine	HHV-6	7.2×10^4	13	Prednisolone 10 mg/day	4	23.8	Erythroderma	1200	49/107
5	64/F	Mexiletine	HHV-6	3.4×10^5	10	Betamethasone 1.0 mg/day	5	17.3	Maculopapular erythema, purpura	3000	100/182
6	44/M	Carbamazepine	HHV-6, HHV-7	7.4×10^3 (HHV-6)	11	Betamethasone 1.0 mg/day	6	14.7	Erythroderma, pustules	7300	91/130
7	62/M	Lamotrigine	HHV-7	IgG (1 : 20) (day 15); IgG (1 : 1280) (day 29)	13	None	7	13.3	Maculopapular erythema	700	28/104
8	32/M	Allopurinol	HHV-6	4.8×10^3	8	None	9	9.9	Maculopapular erythema	2200	52/304
9	56/M	Cyanamide	HHV-6	2.4×10^4	4	None	10	15.6	Maculopapular erythema	3200	101/119
10	57/F	Salazosulfapyridine	HHV-6	2.6×10^3	10	Prednisolone 10 mg/day	10	7.5	Erythroderma, pustules	5800	257/383
11	13/F	Carbamazepine	HHV-6	2.0×10^4	2	None	13	7.8	Maculopapular erythema	2100	124/295
12	36/F	Lamotrigine	HHV-6	1.4×10^5	6	None	13	6.2	Erythroderma	2400	40/108

HHV, herpesvirus. *Loads are number of virus copies/mL. Maximum value in the category of eosinophil and alanine aminotransferase/aspartate aminotransferase during the course of DIHS/DRESS.

Table 2 Profiles of patients with graft-versus-host disease after allogenic stem cell transplantation.

Underlying disease	Transplant type	Pre-transplant conditioning	Viral reactivation	Viral DNA loads* (of whole blood)	Time from disease onset to skin biopsy, days	FoxP3+/CD3+ cells in skin lesions,%	Grade of GVHD
ALL	CBCT	TBI, FLU, BU	HHV-6	1.6×10^4	7	9.5	I
MDS	PBSCT	TBI, FLU, CPA, Mesna	HHV-6, CMV	5.2×10^3	3	4.2	I
MDS	CBCT	TBI, FLU, CPA, Mesna	HHV-6, CMV	8.0×10^4	3	3	I
AML	PBSCT	TBI, FLU, BU	HHV-6	9.2×10^3	2	2.5	IV
MDS	PBSCT	FLU, BU	CMV	4.4×10^3	5	4.8	II
ALL	CBCT	TBI, CPA, VP-16	HHV-6	1.2×10^3	6	8.2	II
ALL	PBSCT	FLU, BU	ND	ND	4	0.7	IV
ALL	BMT	TBI, CPA, BU, Mesna	ND	ND	29	3.3	III
ALL	BMT	TBI, L-PAM	ND	ND	27	0.6	IV
MM	PBSCT	L-PAM, BTZ	HHV-6	3.4×10^3	6	4.2	II
CML	BMT	TBI, FLU, BU, ATG	HHV-7	8.4×10^3	3	9.8	I
AML	CBCT	FLU, BU, Ara-C	HHV-6	7.2×10^3	4	6.7	I

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; Ara-C, cytosine arabinoside; ATG, antithymocyte globulin; BMT, bone marrow transplantation; BTZ, bortezomib; BU, busulfan; CBCT, cord blood cell transplantation; CLL, chronic lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CPA, cyclophosphamide; FLU, fludarabine; GVHD, graft-versus-host disease; L-PAM, L-phenylalanine monohydrochloride; MDS, myelodysplastic syndrome: MM, multiple myeloma; ND, no data; PBST, peripheral blood cell stem cell transplantation; TBI, total body irradiation; VP-16, etoposide. *Loads are number of virus copies/mL.

correlation coefficient was used to evaluate the correlation between the FoxP3+ Treg/CD3+ T-cell ratio in lesional skin and the number of days from onset. $P < 0.05$ was considered statistically significant.

Results

Histopathological examination

Histopathological examination of skin biopsies obtained from the erythematous maculopapular rashes of patients with DIHS/DRESS showed perivascular lymphocytic infiltration with eosinophils (8 cases; 66.7%), interface dermatitis with vacuolar degeneration (2 cases; 16.7%) and spongiotic dermatitis with vacuolar degeneration (2 cases; 16.7%). Skin biopsies from rashes in patients with acute GVHD were graded according to the criteria[14,15] and showed vacuolar degeneration (histological grade I; 6 cases; 50%) and spongiosis with apoptotic cells (histological grade II; 6 cases; 50%). None of the cases showed a cleft between the epidermis and dermis (histological grade III or IV). Tissue from MDE mainly exhibited perivascular lymphocytic inflammation, occasionally with eosinophils.

Increased FoxP3+ Treg/CD3+ T-cell ratio in the skin lesions of DIHS/DRESS

The FoxP3+ Treg/CD3+ T-cell ratio was significantly higher in DIHS/DRESS rashes than in GVHD and MDE tissue (Figs 1 and 2), but the ratio in GVHD was not significantly different from that in MDE. In skin biopsy specimens from GVHD rashes and MDEs, we found small numbers of FoxP3+ Tregs. By contrast, CD4+/CD3+ and CD8+/CD3+ T-cell ratios in the skin lesions were similar for the three groups (Figs 1 and 2). The numbers of CD3+ T cells per 5 high-power fields in skin biopsies of those patients were also not significantly different.

Relationships between FoxP3+ Tregs/CD3+ T cells and the period from onset

Figure 3 shows the relationships between the ratio of FoxP3+ Tregs/CD3+ T cells in the lesional skin and the number of days from disease onset. None of the patients with DIHS/DRESS in this study had received any major treatment such as high-dose corticosteroid before the skin biopsies were taken. The FoxP3+ Treg/CD3+ T-cell ratio was positively correlated with the number of days from disease onset during the acute phase in DIHS/DRESS, but there was no correlation in either GVHD or MDE.

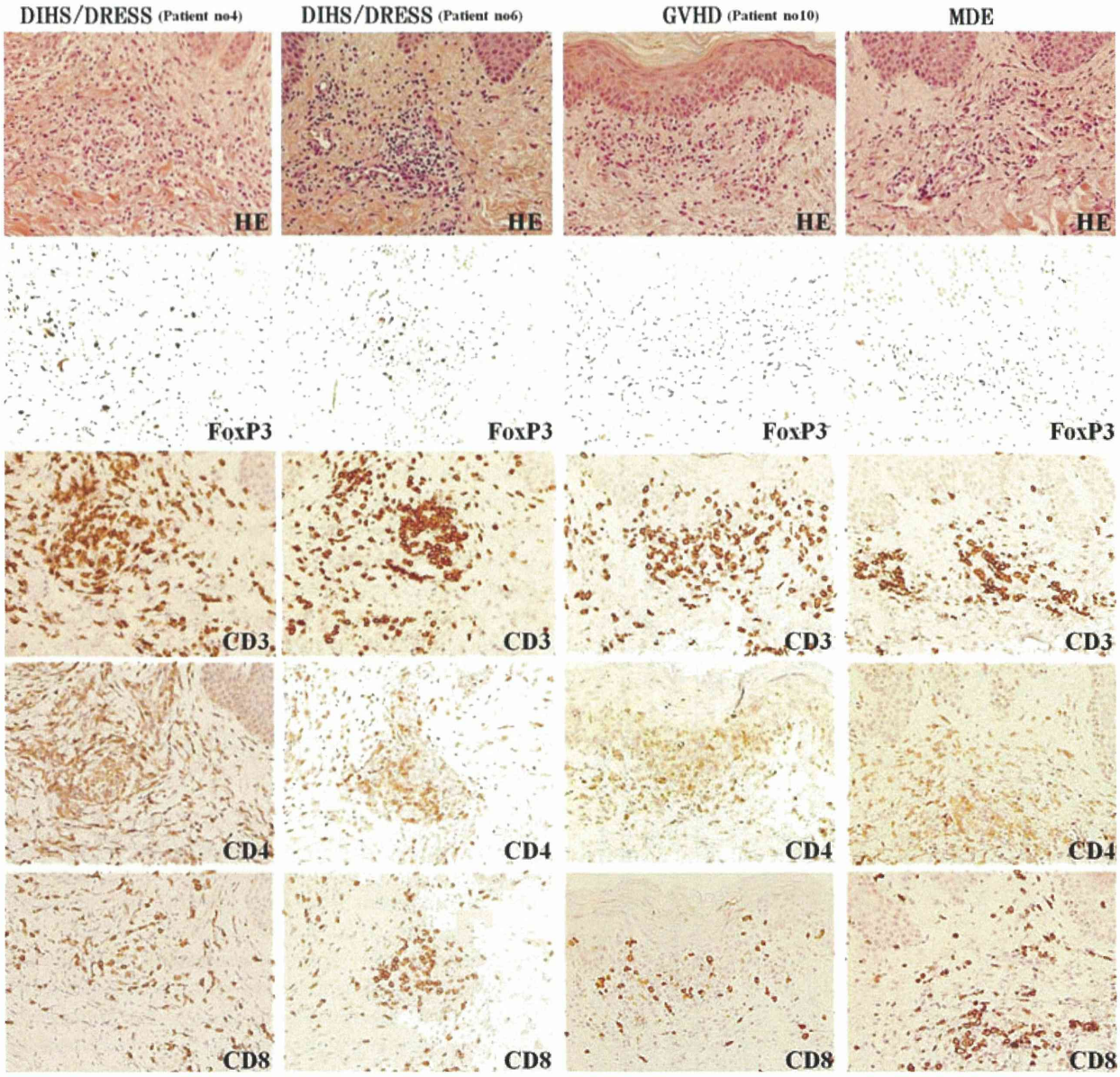

Figure 1 Expression of FoxP3+, CD3+, CD4+ and CD8+ T cells in drug-induced hypersensitivity syndrome (DIHS/DRESS), graft-versus-host disease (GVHD) and maculopapular drug eruption (MDE). Skin biopsies from patients with DIHS/DRESS showed a high number of FoxP3+ T cells in the epidermal–dermal junction and upper dermis compared with those in GVHD and MDE. Sections were counterstained with haematoxylin, and images show representative serial sections from the same lesion of a patient with each disease (original magnification × 200). Patient numbers correspond to those in the tables.

Discussion

Although DIHS/DRESS and GVHD can have similar presentations, there are a some clinical and histological differences between them. The cutaneous presentation of DIHS/DRESS often involves a maculopapular rash or erythroderma, but not blister formation or erosion. The common pathological findings of DIHS/DRESS are superficial perivascular lymphocytic infiltration with extravascular eosinophils, but histologically, severe liquefaction degeneration of the basal layer or epidermal necrosis is rarely found. By contrast, GVHD often presents with blister formation and erosion, and histologically shows lichenoid reaction with epidermal necrosis and/or epidermolysis.

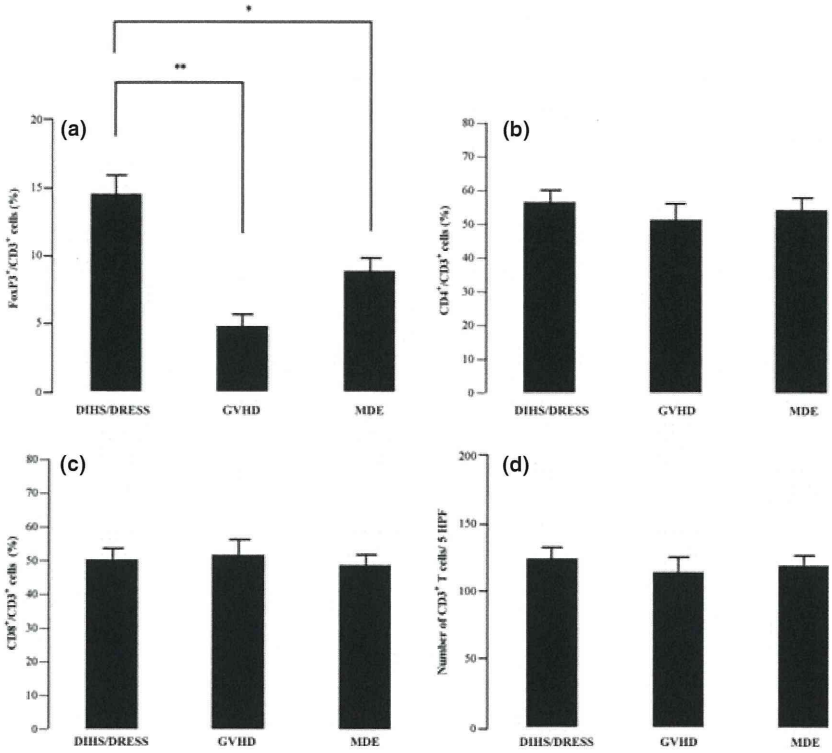

Figure 2 Ratios of FoxP3+ regulatory T cells, CD4+ T cells and ratio of CD8+ T cells to CD3+ T cells in paraffin wax-embedded biopsies taken from patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS; $n = 12$), graft-versus-host disease (GVHD; $n = 12$) and) and maculopapular drug eruption (MDE; $n = 18$). (a) In DIHS/DRESS, a high ratio of FoxP3+ T cells per 100 CD3+ T cells was observed. (b,c) The ratios of CD4+/CD3+ and CD8+/CD3+ T cells infiltrating into the lesional skin of DIHS/DRESS were not statistically different from those in GVHD and MDE. (d) Numbers of infiltrating CD3+ T cells were quite similar in DIHS/DRESS, GVHD and MDE (*$P < 0.05$, **$P < 0.01$).

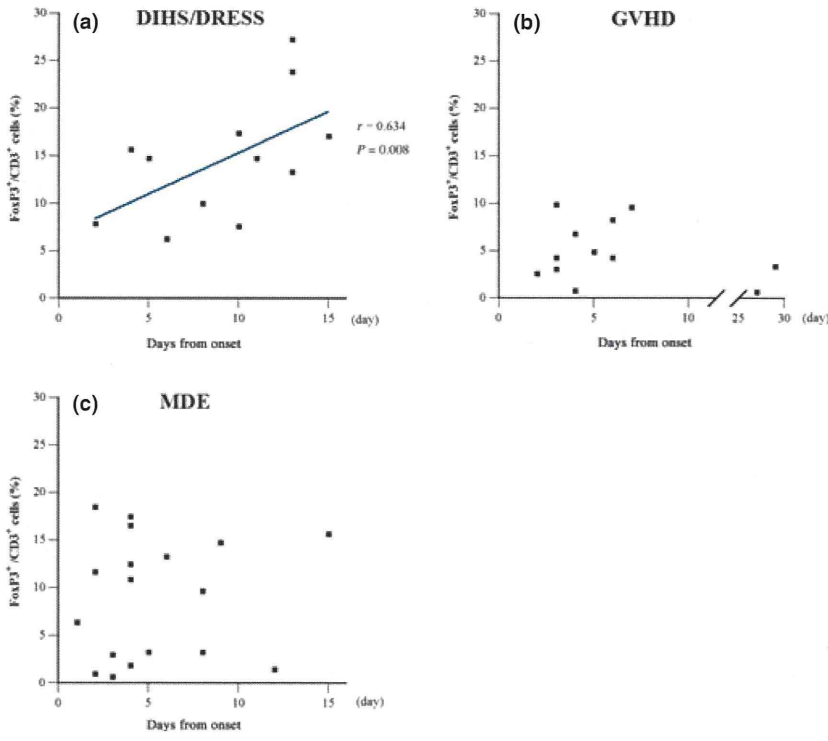

Figure 3 There was a correlation between the FoxP3+ Treg/CD3+ T-cell ratio and the time from disease onset in skin biopsies from patients with drug-induced hypersensitivity syndrome/drug rash with eosinophilia with systemic symptoms (DIHS/DRESS). The ratio of FoxP3+ Treg cells/CD3+ T cells in skin biopsies was correlated with the period from the disease onset only in DIHS/DRESS. MDE, maculopapular drug eruption.

Previous research on the dynamics of skin-infiltrating Tregs in GVHD showed that a decreased number of skin-infiltrating Tregs was associated with severity of GVHD;[17] however, another study showed that Tregs increased with degree of inflammation and grade of GVHD.[18]

Patients with DIHS/DRESS in the acute stage were found to exhibit increased frequencies of Tregs and gradual loss of their function after resolution in peripheral blood mononuclear cells (PBMCs).[19] However, there have been no studies about the dynamics of skin-infiltrating Tregs in DIHS/DRESS. Therefore, we focused on the dynamics of infiltrating Tregs in the skin lesions of these diseases, and found considerable differences between DIHS/DRESS and GVHD.

In the current study, the FoxP3+ Treg cell/CD3+ T-cell ratio was significantly higher in lesions from DIHS/DRESS than in those from GVHD and MDE, whereas the numbers of CD3+ T cells infiltrating into the skin lesions were similar in all three conditions (Figs 1 and 2). We also found that the ratio was positively correlated with the number of days from disease onset during the acute phase of DIHS/DRESS (Fig. 3). However, each dot in Fig. 3 represents the FoxP3+/CD3+ ratio from different patient samples, so the data does not show sequential data from individual patients, and thus results must be interpreted with caution. By contrast, the ratios of CD4+CD3+ T cells and CD8+CD3+ T cells in cutaneous lesions were similar for DIHS/DRESS, GVHD and MDE (Fig. 2). These findings suggest that clinical and histological differences between DIHS/DRESS and GVHD may result from differences in the frequency of FoxP3+ Tregs infiltrating into the skin lesions of these diseases. Tregs play a significant role in suppression of various diseases, including allergic responses, autoimmune and infectious disease, and cancers.[20,21] Accordingly, it is likely that an increased number of FoxP3+ T cells infiltrating into DIHS/DRESS skin lesions can protect the epidermis from severe damage compared with that in GVHD skin lesions.

Conclusion

In conclusion, the present study suggests that, despite many similarities, the dynamics of Tregs are different between DIHS/DRESS and GVHD in skin lesions, and that this difference may exert a considerable influence on the development of skin presentations in the two diseases.

What's already known about this topic?

- There are close similarities between DIHS/DRESS and GVHD, including HHV-6 reactivation, skin eruption, and autoimmune disease-like complications.
- However, there are also some clinical and histological differences between these two conditions.
- There are conflicting reports about the dynamics of skin-infiltrating Tregs in GVHD: severity of disease has been associated with both a decreased and an increased number of skin-infiltrating Tregs.
- Patients with DIHS/DRESS exhibit increased frequencies of Tregs in PBMCs at the acute stage; however, the dynamics of skin-infiltrating Tregs in DIHS/DRESS are currently unknown.

What does this study add?

- In the current study, levels of FoxP3+ Tregs were significantly higher in the skin lesions of DIHS/DRESS than in those of GVHD.
- The FoxP3+ Treg cell/CD3+ T-cell ratio was positively correlated with the number of days from disease onset during the acute phase of DIHS/DRESS, but not in GVHD or MDE.

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SHORT COMMUNICATION

Stevens-Johnson Syndrome Associated with Mogamulizumab-induced Deficiency of Regulatory T cells in an Adult T-cell Leukaemia Patient

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Stevens-Johnson syndrome (SJS) is a rare but extremely severe drug-induced eruption characterised by widespread necrosis or apoptosis of the epidermis (1). The main causal factor for SJS is an aberrant activation of CD8 T cells. Although the dysfunction of the regulatory T cells (Treg) is considered a possible cause of excess CD8 T cells activation (2), the actual significance of Tregs *in vivo* remains unknown.

CASE REPORTS

A 54-year-old man with relapsed adult T-cell leukaemia-lymphoma (ATL) (stage IVB) was referred to our clinic with multiple erythema and small red papules on his lower legs (Fig. 1a). Eight days earlier (day 0) he had started weekly treatment with mogamulizumab, a humanised anti-CC chemokine receptor 4 (CCR4) monoclonal antibody that depletes $CCR4^+$ tumour cells in ATL patients. Histological examination of the papules exhibited inflammatory cell infiltration in the perivascular and interface between the dermis and the epidermis (Fig. 2a). $CD8^+$ cells were mainly located in the perivascular region, and forkhead box P3 $(FOXP3)^+$ Tregs existed in the interface (Fig. 2b, c). Necrosis of epidermal cells was not detected. We diagnosed the skin rash as a drug-induced eruption by mogamulizumab. As topical difluprednate treatment did not control the skin rash, we discontinued the third course of mogamulizumab treatment, and started 30 mg of oral prednisolone treatment on day 12.

Although the erythema and papules temporarily improved after the systemic steroid therapy, the clinical manifestations spread over his trunk and extremities (Fig. 1b) on day 28 accompanied by severe conjunctivitis, erosion and swelling of oral mucosa (Fig. 1c), and high fever. Histological examination on day 28 revealed severe epidermal cell necrosis and vacuolar changes with significant numbers of $CD8^+$ cells in the interface in accord with the absence of Tregs (Fig. 2d–f). Flow cytometry analysis revealed that Tregs in blood had disappeared by day 17 (Fig. 3a, b). We diagnosed the patient as SJS induced by mogamulizumab. We initiated pulse therapy with methylprednisolone (500 mg/day × 3 days) plus tacrolimus (1.5 g/day × 2 days), followed by methylprednisolone treatment (70 mg/day). After these treatments, the skin rash gradually improved. We carefully tapered the dose of methylprednisolone to 30 mg/day. Even after the discontinuation of mogamulizumab

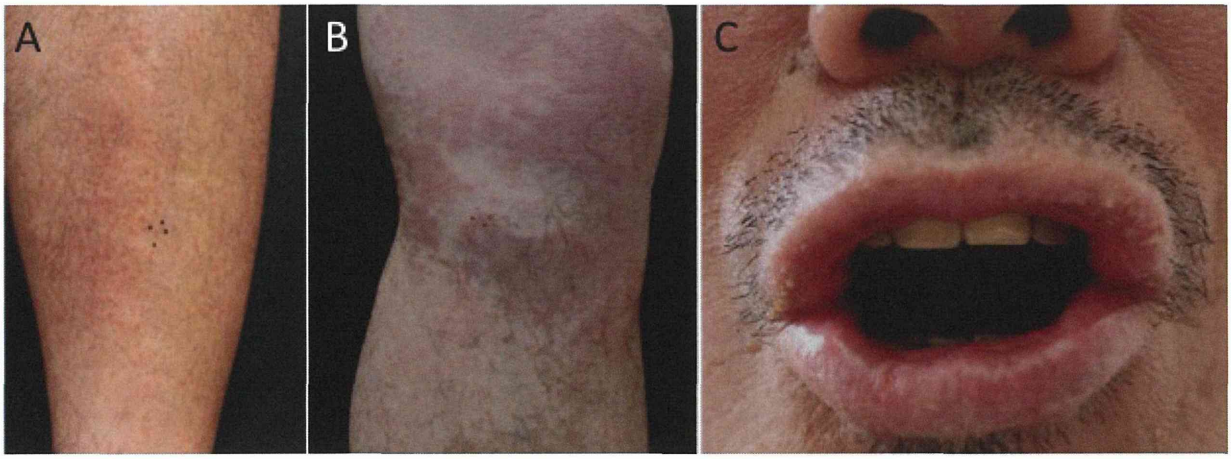

Fig. 1. Multiple erythema and small papules on day 8 (a). Erythema multiforme-like erythema on extremities (b), and erosion and swelling of oral mucosa on day 28 (c).

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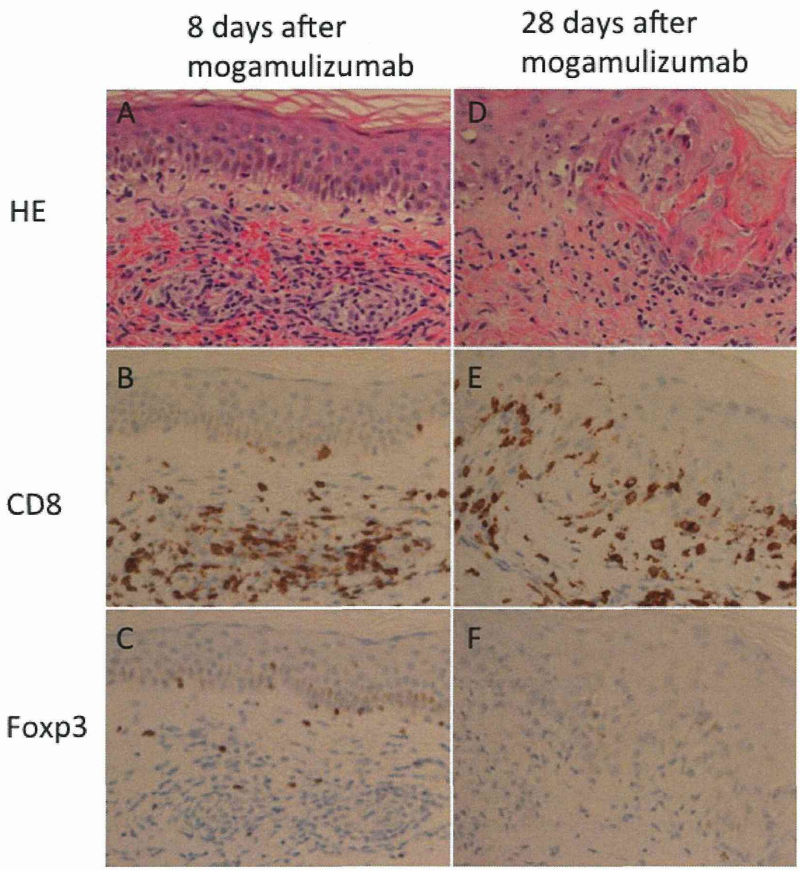

Fig. 2. Histological and immunohistochemical (IHC) findings 8 (a–c) and 28 days (d–f) after mogamulizumab treatment. (a, d) Haematoxylin and eosin staining. IHC for CD8 (b, e) and FOXP3 (c, f). Original magnification: ×40.

therapy, the patient achieved a complete remission in ATL. He was discharged on day 81.

DISCUSSION

Mogamulizumab was approved in Japan in March 2012 as a novel therapy for relapsed or refractory ATL, because tumour T cells of ATL patients are $CCR4^+$ in nearly 90% of cases (3). Although it significantly improves the clinical symptoms in ATL patients, it sometimes induces severe adverse effects of the skin, such as SJS and toxic epidermal necrolysis (4). In the current case, the development of SJS was inversely correlated to the presence of Tregs in the skin. Tregs were probably depleted by mogamulizumab, because CCR4 is highly expressed on Tregs as well as on ATL tumour cells (4, 5). Although more cases are to be analysed, our case may provide *in vivo* evidence that absence of Treg functions in skin is the primary cause of SJS, at least during the treatment with mogamulizumab.

It has been reported that the skin lesion by mogamulizumab usually developed after the fourth or subsequent infusion (4, 6), while the skin lesion in our case developed just after the second infusion. Thus far, it remains unknown what kind of factors determine the onset or severity of adverse skin reaction by mogamulizumab (6). Further research is required to reveal the mechanism.

The authors declare no conflict of interest.

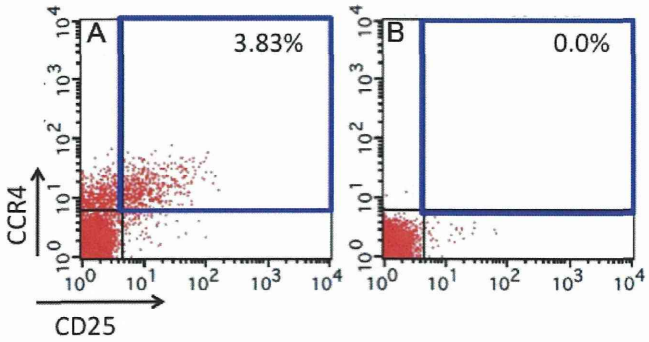

Fig. 3. Flow cytometry analysis for $CCR4^+CD25^+CD4^+CD3^+$ non-tumour cells in peripheral blood before (a) and 17 days (b) after the mogamulizumab therapy. Data are gated on $CD4^+CD3^+$ cells.

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